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Standard Operating Procedure

Lethal Fish Processing

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1 ANAESTHETIZING

1.1 Field Procedures

A variety of samples and measurements can be collected when conducting a fish survey. These can be obtained either through lethal or non-lethal means. Fish retained for lethal sampling must be euthanized by either a decisive blow to the head or by anaesthesia until respiration has stopped.

When anaesthetizing fish for lethal sampling, set up the anaesthetic bath prior to extensive fish handling. The equipment required for the anaesthetic bath includes:

- an adequately-sized tub,
- fresh water,
- clove oil,
- ethanol, and
- a measuring cup/syringe.

Set-up and use the anaesthetic bath as follows:

a. Add ethanol and clove oil (in a ratio of 9:1) to site water (for a final anaesthetic concentration of 60-ppm or 0.06 mL per litre of water).

b. Place the fish in the anaesthetic and wait until respiration has stopped.

2 MERISTICS

2.1 Length

Three types of length measurements can be taken from a fish - total, fork, and standard (Figure 1). Total length is measured from the tip of the snout to the dorso-ventrally compressed lobes of the caudal fin. Fork length is the distance between the tip of the snout and the middle of the caudal fin. Standard length is the distance between the tip of the snout of the fish and the middle of the caudal peduncle. When taking a length measurement, ensure that the fish is lying flat on the measuring board, and that its snout is at the end of the board.

If a measuring board is not available, rulers, meter sticks, or tape measures can be used. Electronic calipers may also be useful for very small fish (i.e., cyprinids or juvenile fish). Record length measurements as precisely as possible.

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Figure 1: **Measurements of Fish Length: Total, Fork, and Standard**

2.2 Body Weight

Fresh whole body weights can be measured using either spring scales or electronic balances, depending on the size of the fish and the desired measurement accuracy. When measuring the whole body weight, make sure the fish is free of extra water on the surface of its body.

Assess the scale or balance accuracy each day using standardized weights and calibrate if necessary. Tare the electronic balance prior to each measurement. Always use a scale or balance that is most accurate for the size of the fish being weighed.

To use a spring scale, insert the hook behind the gill cover below the lower jaw or attach the clamp to the base of the caudal peduncle. Hold the scale at eye level and read the weight as precisely as possible. To use an electronic balance, place the fish in a weigh boat in the centre of the plate (tare the scale with the weigh boat prior to weighing). Wait for the reading to stabilize and then record the measurement.

2.3 Abnormalities

Fish are assessed for abnormalities by evaluating the frequency of deformities, erosions, lesions, and tumors (DELT survey; Sanders et. al. 1999[1]) as well as external parasites, and scale disorientation. Record all instances of abnormalities on the field sheets.

2.4 Sex Determination and Gonad Weight

To determine the sex of the fish, create an incision (using round-nosed scissors) on the ventral surface of the body from a point immediately anterior to the anus toward a point immediately posterior to the pelvic fins. Gonads are located dorso-laterally in the anterior end of the visceral cavity. Ovaries appear whitish to greenish to golden brown and have a granular texture, while testes are smooth and creamy white. Record the sex of the fish on field sheets. Remove gonads from the surrounding tissue using forceps and/or round-nosed scissors. Weigh the whole gonads using an electronic balance to the nearest 0.001 g. If the gonads are required for chemical analysis, transfer them to an appropriate pre-labelled sample jar or bag and place in a freezer. If the ovaries are required for fecundity and egg weight analysis, place them in a pre-labelled sample jar and preserve in a solution of 10% formalin. Ensure that a sufficient volume of solution has been added to fully cover the ovaries. Check the samples daily prior to sending to the laboratory to ensure proper preservation (i.e., ensure samples have not started to change colour/decay due to insufficient formalin coverage). Formalin is a carcinogen and an irritant to workers, so protective gloves, respiratory gear, and eye protection are required when using formalin.

2.5 Liver Weight

If the fish is not already opened, create an incision (using round-nosed scissors) on the ventral surface of the body from a point immediately anterior to the anus toward a point immediately posterior to the pelvic fin. The liver is located in the anterior visceral cavity behind the heart and ahead of the stomach. It will be pink to dark red to brown in colour, and may consist of several lobes. When removing the liver, ensure that all parts are collected while being careful to exclude obvious fat deposits and the gallbladder. Weigh the liver to the nearest 0.001 g on an electronic balance. If the liver is required for further analysis (e.g., tissue chemistry), transfer it to an appropriate pre-labelled sample jar or bag and place in a freezer.

[1] Sanders, R.E., R.J. Miltner, C.O. Yoder, and E.T. Rankin. 1999. The use of external deformities, erosion, lesions, and tumors (DELT anomalies) in fish assemblages for characterizing aquatic resources: A case study of seven Ohio streams. In: T.P. Simon (Ed.), pp: 225-246, Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, FL.

3 AGE STRUCTURES

3.1 Selecting Age Structures

If possible, collect two appropriate age structures from each fish sampled (Table 1). Details related to the collection of each type of age structure are provided in the sections that follow.

Table 1: **Fish Species and their Respective Primary and Secondary Age Structures**

Species	Non-lethal		Lethal*	
	Primary Age Structures	Secondary Age Structures	Primary Age Structures	Secondary Age Structures
Bass	dorsal spines	scales	otoliths	scales
Bullhead	pectoral fin rays	-	otoliths	pectoral spine
Burbot	-	-	otoliths	-
Catfish	pectoral fin rays	-	otoliths	pectoral spine
Char	pelvic fin rays[1]	scales	otoliths	scales
Chub	pelvic fin rays	scales	otoliths	scales
Cisco	pelvic fin ray	scales	otoliths	scales
Cod	-	-	otoliths	-
Crappie	pelvic fin rays	scales	otoliths	scales
Dace	pec/pel fin rays	scales	otoliths	pec/pel fin rays
Darter	pec/pel fin rays	scales	otoliths	pec/pel fin rays
Flounder	-	-	otoliths	-
Grayling	pec/pel fin rays	scales	otoliths	pec/pel fin rays
Herring	pelvic fin ray	scales	otoliths	scales
Logperch	pec/pel fin rays	scales	otoliths	pec/pel fin rays
Minnow	pec/pel fin rays	scales	otoliths	pec/pel fin rays
Perch	dorsal spines	scales	otoliths	scales
Pike	pec/pel fin ray	scales	cleithra	scales
Redhorse	pectoral fin ray	scales	pectoral fin ray	scales
Salmon	pelvic fin ray	scales	otoliths	scales
Sculpin	-	-	otoliths	-
Shiner	pec/pel fin rays	scales	otoliths	pec/pel fin rays
Smelt	-	-	otoliths	-
Stickleback	-	-	otoliths	-
Sucker	pectoral fin ray	scales	pectoral fin ray	otoliths, scales
Sunfish	dorsal spines	scales	otoliths	dosal spines, scales
Trout	pec/pel fin ray	scales	otoliths	scales
Walleye	dorsal spines	scales	otoliths	scales
Whitefish	pec/pel fin ray	scales	otoliths	scales

[1] take the first 3 to 5 lead rays as tight to the body as possible
* whenever possible multiple tissues should be collected (multiple tissues would in most cases include otoliths, fin-rays/spines and scales for most species [scales on younger fish <5 years old]).

3.2 Scales

Scales are to be taken from different parts of the fish depending on species; check and confirm the appropriate location for each species (Figure 2). To collect the sample:

a. Orient the fish so that scales will be collected from the left side of the body.

b. Wipe excess mucus and or dirt from the area with the dull side of a knife.

c. With the tip of the knife, pull approximately six to ten scales from the body by gently scraping in a head-to-tail direction. Do not take scales from areas of the body where previous damage has occurred and scale regeneration is evident.

d. Place the scales on wax paper, separate them on the paper, fold the paper, and place it into a pre-labelled envelope.

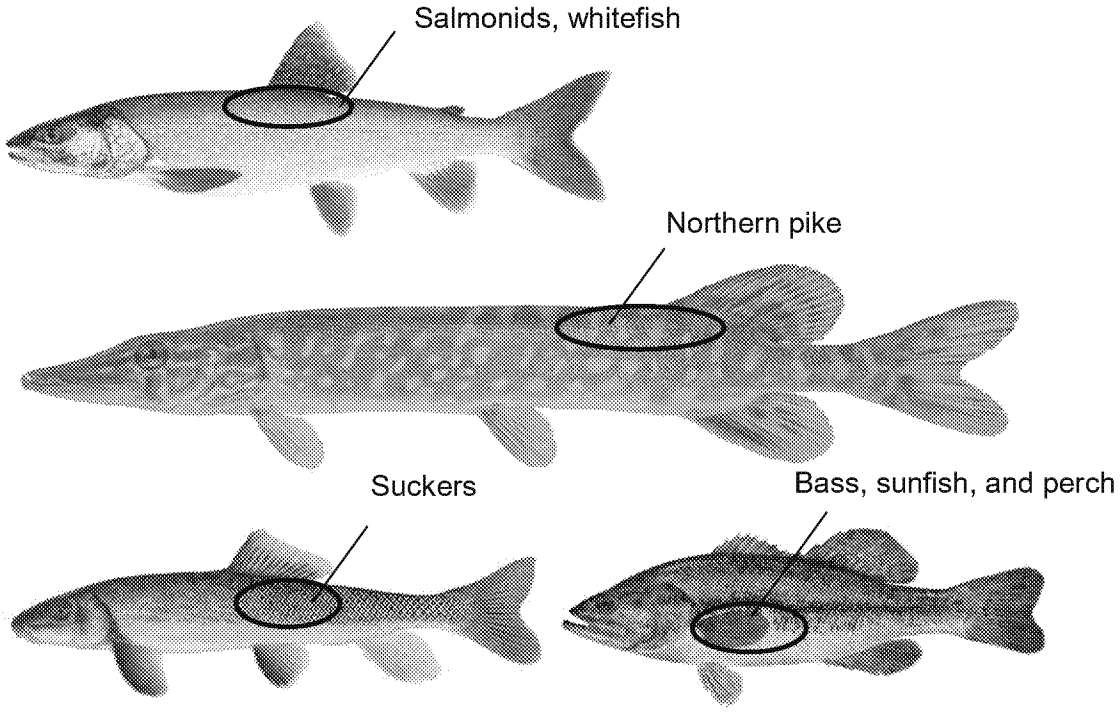

Figure 2: **General Scale Sampling Locations (Modified from Ontario Ministry of Natural Resources and Laurentian University Fish Processing Manual)**

3.3 Otoliths

Fish have three pairs of otoliths: the sagittae, lapilli, and asterisci. The sagittae are largest and most commonly sampled for age determination. They are located behind the eyes, approximately vertically level with the eyes. The lapilli and asterisci (smallest of the three) are located within the semicircular canals.

For age determination of small fish (< 10 cm), remove the entire head (cutting directly behind pectoral fins) and place in a pre-labelled bag. Freeze the sample as soon as possible and send to laboratory.

For large fish, otoliths must be removed prior to submission to the laboratory, using the following procedures:

a. The otoliths (sagittae) can be accessed from the bottom of the jaw.

b. Hold the fish by the head, ventral side up and cut the isthmus (the skin under the lower jaw) to obtain access to the roof of the mouth cavity.

c. Peel back the gills or the skin/tongue and clean the otic capsule/bulla.

d. Cut the otic capsule/bulla at the base with cutters and break it open.

e. Carefully remove the otoliths with tweezers and wipe off any excess tissue.

f. Wrap the otoliths in wax paper and place them into a pre-labelled envelope or sample bag. It is not necessary to freeze the otolith.

3.4 Spines and Fin Rays

Collect a spine or fin ray as follows:

a. Select the appropriate spine or fin ray based on the fish species (see Table 1). Typically, the longest spine/ray nearest the anterior end of the fish is preferred.

b. Take more than just one dorsal spine, to give the laboratory a choice of structures to provide the best age estimate.

c. Using a sharp knife, separate the dorsal spines or fin ray that you are sampling from the fin and cut around the base of the knuckle.

d. Remove the spine/ray with a knife or scissors by cutting under the knuckle.

e. Strip the spine/ray of as much tissue as possible and remove it cleanly (being sure to include the base/knuckle) from the fish.

f. Wrap the spine/ray in wax paper and insert it into a pre-labelled envelope or sample bag. If the samples are not completely free of muscle/skin tissue, store the samples in a freezer until submission to the laboratory.

4 MUSCLE TISSUE FOR CHEMICAL ANALYSIS

4.1 Sample Collection

Prior to tissue sampling, wash and disinfect all sampling tools and cutting boards. This is particularly important when collecting tissue samples for chemical analyses. If possible, conduct all dissections in a clean, laboratory environment. When not possible, make sure the dissection area can be kept as sanitary as possible.

Select only intact fish for tissue sampling. Avoid any fish with lacerations, as it can expose the fish to additional contamination sources (e.g., sampling gear).

A fillet of muscle tissue is collected for tissue chemistry analysis, as follows:

a. Process the fish as soon as possible after collection.

b. Euthanize the fish by a decisive blow to the head or by using an anaesthetic bath until respiration has stopped (see Section 1).

c. Begin by making a shallow cut through the skin on either side of the dorsal fin, from the top of the head to the base of the tail.

d. Then, make a cut behind the entire length of the gill cover cutting through the skin and flesh to the bone.

e. Make a shallow cut along the belly from the base of the pectoral fin to the tail. The first cut is made from behind the gill cover to the anus, and then continued down on both sides of the anal fin. Take care to avoid puncturing an internal organ while filleting the fish, which can contaminate the tissue sample.

f. Remove the fillet by cutting from the incision behind the gill cover, along the spine, to the tail. Include the belly flap in the fillet.

g. Carefully remove the skin and any bones remaining in the fillet.

h. Weigh the fillet on an electronic balance and record the weights to the nearest gram. Place the sample into a pre-labelled plastic bag. Store all muscle samples on ice (while working in the field) and then place in a freezer as soon as possible.

5 FROM THE 2018 TO 2020 KOOCANUSA STUDY DESIGN

5.1 General Information

Fish tissue sampling in the reservoir will include collection of sport fish (e.g., bull trout) muscle using non-lethal methods (i.e., muscle plug). Peamouth chub, redside shiner, and northern pikeminnow will also be sampled lethally for muscle and ovary tissue chemistry.

5.2 Sample Collection

5.2.1 Fish Tissue

The targeted species, the number of samples collected, and the timing of collection for the fish tissue chemistry assessment will be as follows:

- sport fish muscle (non-lethal muscle plugs) collection from up to eight individuals per species in each of three study areas in 2018;
- peamouth chub and redside shiner ovary and muscle collection from up to 10 females per study area in April 2018 to 2020; and
- northern pikeminnow ovary and muscle collection from up to 10 females per study area in June 2018.

The sport fish collection will target species previously collected in Koocanusa Reservoir (i.e., bull trout, kokanee, mountain whitefish, rainbow trout, westslope cutthroat trout, and yellow perch). Burbot will not be a target species for muscle sampling based on concerns about burbot abundance[2] and the cultural importance of this fish species to the KNC. If burbot are caught, they will be immediately released.

Fish that will be sacrificed for tissue sampling include peamouth chub, northern pikeminnow, redside shiner, and yellow perch. Sampling methods will be consistent with those described in the first four sections of this document.

Samples will be stored frozen pending shipment to the laboratory for analysis.

5.3 Laboratory Analysis

5.3.1 Fish Age

Fish tissues collected for age analysis will be submitted to a qualified laboratory for analysis. Otoliths will be prepared and read under a compound microscope using transmitted light. For

[2] In recent years, lower Kootenay burbot populations were designated as critically imperiled and red-listed, meaning potentially extirpated, endangered, or threatened

each structure, the age and edge condition will be recorded along with a confidence rating for the age determination. For the purpose of quality assurance/quality control (QA/QC), approximately 10% of samples will be assessed by a second individual at the laboratory.

5.3.2 Tissue Chemistry

Fish tissue samples for chemical analysis will be submitted to a qualified laboratory consistent with Ministry of Environment and Climate Change Strategy laboratory guidance as specified in Permit 107517 (Province of BC 2015[3]).

Samples will first be freeze-dried for determination of moisture content and then analyzed for metals (including mercury) using high-resolution inductively couple mass spectrometry (HR-ICP-MS). Results will be reported on a dry weight basis, along with moisture content (based on the difference between wet and freeze-dried sample weights) to allow conversion to wet-weight values. Accuracy and precision of data will be judged based on ability to achieve minimum laboratory reporting limits, replicate analysis of a minimum of 10% of samples, as well as comparison to certified reference materials.

[3] Province of British Columbia. 2015. British Columbia Environmental Laboratory Manual (complete). Available from https://www2.gov.bc.ca/gov/content/environment/research-monitoring-reporting/monitoring/laboratory-standards-quality-assurance/bc-field-sampling-manual. Accessed January 29, 2019.